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(54) Title: NOVEL ANTIMICROBIAL RESISTANCE BLOCKING COMPOSITIONS

(57) Abstract: This invention is concerned with antimicrobial compositions that control or prevent resistance to antimirobial effectiveness, more particularly to combinations of topical antimicrobial agents with antimutagenic or antioxidant agents that block intrinsic or acquired bacterial resistance.

NOVEL ANTIMICROBIAL RESISTANCE BLOCKING COMPOSITIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention is concerned with antimicrobial compositions that control or prevent resistance to antimicrobial effectiveness, more particularly to combinations of topical antimicrobial agents with agents that block development of intrinsic or acquired bacterial resistance.

2. Related Art

Among several diseases, opportunistic infectious diseases are leading causes of death among patients. Reputed researchers also suspect a link between certain of these infectious diseases and life threatening diseases like cancer, heart disease, asthma, ulcers and allergies that were not traditionally considered as due to microbial causes. In the United States, an estimated 1.2 million patients each year develop nosocomial infections that are resistant to conventional antibiotics, and approximately 60,000 deaths occur each year adding an estimated $4.5 billion to the annual cost of health care in the US alone. This recent emergence of widespread antimicrobial resistance is a major public health problem locally as well as globally.

Antimicrobial resistance by microbes is considered to be intrinsic or acquired. Intrinsic resistance is a natural property of microorganisms. It is often the result of the cell wall or outer membrane restricting entry and intracellular accumulation of antimicrobial agents (including efflux mechanisms). Also, resistance may be due to biofilms that are inherently resistant to antimicrobials due to sticky conglomerates of bacteria.

Acquired resistance is the result of changes in bacterial genetic material due to mutations or the acquisition of extra chromosomal genetic material. Recent awareness of acquired genetic resistance to triclosan and related antimicrobials through missense mutations of the FAB1 gene suggests that the widespread use of these types of compounds will lead to increased appearance of resistant microbes that will compromise the present usefulness of triclosan or similar antimicrobial compounds as well as antibiotics used systemically. Researchers clearly suggest that the development of second generation FAB1 inhibitors would help limit resistance. Certainly, the pharmaceutical industry is working to develop new means of combating resistant pathogens. Diagnostics manufacturers also have an opportunity to develop products that aid treatment by detecting specific drug resistant infections. In

addition to focusing on discovering and developing newer antimicrobial compounds, it is very important to better utilize the existing classes of compounds with altogether new strategies to control, prevent or eradicate the resistant organisms. Though frequent handwashing might remove transient flora, dirt and oils from the skin it is becoming increasingly important to formulate unique products with unique technological characteristics that are capable of enhancing antimicrobial effectiveness by removing/killing antibiotic resistant strains while preventing the emergence of resistant strains from susceptible strains.

Resistance to antimicrobial agents is now a serious clinical and a major public health problem in the U.S. The international spread of pathogenic organisms clearly demands a global strategy. New drugs are unlikely to appear soon enough and in sufficient number to solve many of these resistance problems. Hence, there is a growing need to understand the factors that lead to evolution of the spread of resistance and to design strategies to maximize the effectiveness of existing and future drugs/products while minimizing the spread of resistance to them. A recent article in the ***American Journal of Infection Control*** (***AJIC***, 26:541-3 (1998)) illustrates the continuing need to examine various facets of the problem and suggested solutions. The antimicrobial compositions of this invention offer one solution to this problem.

SUMMARY OF THE INVENTION

The present invention relates to a topical antimicrobial composition comprising:

(a) a topical antimicrobial agent; and

(b) an antimicrobial resistance blocking effective amount of at least one antimutagenic and/or antioxidant agent.

In one embodiment, the topical antimicrobial is triclosan and the antimutagenic and/or antioxidant compound is selected from the group consisting of substituted and unsubstituted pyrithione-containing compounds; coumarins; pseudopeptides; indazoles; antioxidants; flavanoids; isoflavanoids including isoflavenes, isoflavanes, isoflavanones and isoglabrene analogs; and mixtures thereof.

Another aspect or embodiment of the invention provides a method for inhibiting bacterial resistance comprising:

(a) incorporating an antimicrobial resistance blocking effective amount of at least one antimutagenic and/or antioxidant agent into a topical antimicrobial agent; and

(b) applying the composition of (a) to a surface containing bacteria.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

As used herein, the term "topical antimicrobial" is intended to describe compounds used to inhibit or kill or otherwise prevent proliferation of microbes. The topical antimicrobial agents are used to treat any type of surface, both mammalian and non-mammalian. In humans, these agents are widely used for treatment of the skin.

Suitable topical antimicrobial agents for use with this invention include nisin, bis-diguanides, chlorhexidine gluconate, chlorhexidine digluconate, chlorhexidine diacetate, chlorhexidine dihydrochloride, polyhexamethylene biguanide, benzalkonium chloride, benzethonium chloride, methylbenzethonium chloride, cetyl pyridinium chloride, triclosan, triclocarban, tribromosilane, amyltricresols, parachlorometaxylenol, phenol, silver, iodine, [nonylphenoxypoly (ethyleneoxy) ethanoliodine] poloxamer-iodine complex, undecoylium chloride, iodine complex, bisquaternary ammonium compounds, polymeric quaternary ammonium compounds, alcohols, cationic polypeptides, organometallic antiseptics, alkyl pyridinium salts, essential oils, and their combinations and derivatives. Likewise, a variety of simple alcohols may function in this regard, including but not limited to, ethanol, propanol, butanol, pentanol, 2-methyl-1-butanol, hexanol, 2-

methyl-1-pentanol, 3-methyl-1- pentanol, 2-ethyl-1-butanol, 3,5,5-trimethyl-1-hexanol, heptanol, octanol, isooctyl alcohol, decanol, dodecanol, tridecanol, tetradecanol and the like.

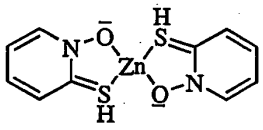
The term "antimutagenic" compound or agent, as used herein, is intended to describe compounds that lessen the extent of antimicrobial resistance. As will be shown hereinafter, not all antimutagenic agents are effective in inhibiting antimicrobial resistance when combined with topical antimicrobial agents.

As used herein, the term "antioxidant" compound or agent is intended to describe oxygen scavenging compounds that prevent or lessen the degree of mutations in genetic material (e.g., DNA), or prevent or lessen the degree of oxidation of poly-unsaturated fatty acids.

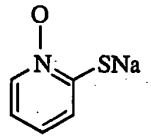
Also as described hereinafter, certain suitable antimutagenic and antioxidant agents are described both by chemical name and chemical structure. Due to the fact that chemical nomenclature for the same structure can vary and for the avoidance of doubt any discrepancy that may arise between a chemically named compound and the corresponding chemical structure shown, the chemical structure is intended to govern the description of the compound.

Examples of suitable antimutagenic and antioxidant compounds useful in this invention include the following compounds and analogs thereof: substituted and unsubstituted pyrithione-containing compounds; coumarins; pseudopeptides; indazoles; antioxidants; flavanoids; isoflavanoids including isoflavenes, isoflavanes, isoflavanones and isoglabrene analogs; and mixtures thereof.

Examples of pyrithione-containing compounds include substituted and unsubstituted pyrithione containing compounds such as monovalent or divalent metallic salts of pyrithione. Preferred are zinc, silver, and sodium salts of pyrithione.



Zinc Pyrithione



Sodium Pyrithione

Examples of suitable coumarins include:

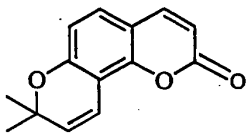
8-(2'2'-Dimethyl-1'H-pyran-7-yl)coumarin;

[(2'2-Dimethyl-3-ß-hydroxy-6-methylene)-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycoumarin;

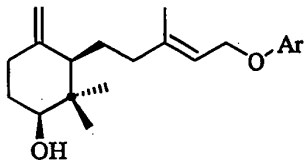
[(2'2,6-Trimethyl-2-oxo-bicyclo)-2,2,1-heptyl]-1-ß-3-methyl-pent-2-enyl-7-oxycoumarin;

7-Cyclohexylmethoxycoumarin; and

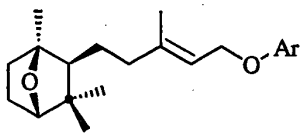
Galbanic Acid.



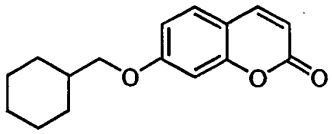
8-(2'2'-Dimethyl-1'H-pyran-7-yl)coumarin



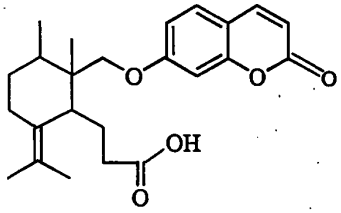
[(2'2-Dimethyl-3-ß-hydroxy-6-methylene)-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycoumarin



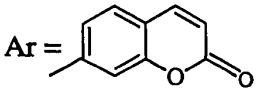
[(2'2,6-Trimethyl-2-oxo-bicyclo)-2,2,1-heptyl]1-ß-3-methyl-pent-2-enyl-7-oxycoumarin



7-Cyclohexylmethoxycoumarin



Galbanic Acid



Preferred coumarins are:

8-(2'2'-Dimethyl-1'H-pyran-7-yl)coumarin; and
Galbanic Acid.

Most preferred coumarins are:

[(2'2-Dimethyl-3-ß-hydroxy-6-methylene)-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycoumarin;
[(2'2,6-Trimethyl-2-oxo-bicyclo)-2,2,1-heptyl]-1-ß-3-methyl-pent-2-enyl-7-oxycoumarin; and
7-Cyclohexylmethoxycoumarin.

Examples of suitable pseudopeptides include:

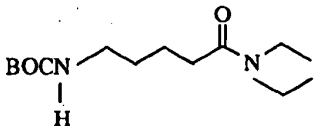
N-Tert-butoxycarbonyloxyaminopentan-N,N-diethylamide;
N-Benzoylalanyl-N,N-diethylamide;
N-Tert-Butoxycarbonyloxyalanyl-N,N-diethylamide;
N-Tert-Butoxycarbonyloxyalanyl-N,N-morphilinoamide;
N-Tert-butoxycarbonyloxyalanyl-N-methyl-N-phenylamide;
4-Benzoylaminobenzoyl-N,N-diethylamide;
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide;
4-Toluenesulfonylaminobenzoyl-N,N-diethylamide;
4-Benzoylaminobenzoyl-N,N-diethylamide;
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide;
5-N-Benzylaminopentanoyl-N,N-diethylamide;
5-N-Cyclopentylaminopentanoyl-N,N-diethylamide; and
5-N-Benzoylaminopentanoyl-N,N-diethylamide.

Preferred pseudopeptides are:

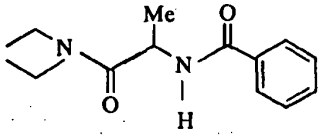
N-Tert-butoxycarbonyloxyaminopentan-N,N-diethylamide;
N-Benzoylalanyl-N,N-diethylamide;
N-Tert-Butoxycarbonyloxyalanyl-N,N-diethylamide;
N-Tert-Butoxycarbonyloxyalanyl-N,N-morphilinoamide;
N-Tert-butoxycarbonyloxyalanyl-N-methyl-N-phenylamide;
4-Benzoylaminobenzoyl-N,N-diethylamide;
4-Toluenesulfonylaminobenzoyl-N,N-diethylamide; and
4-Benzoylaminobenzoyl-N,N-diethylamide.

Most preferred pseudopeptides are:

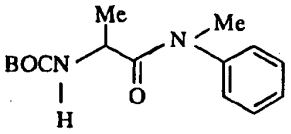
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide;
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide;
5-N-Benzylaminopentanoyl-N,N-diethlamide;
5-N-Cyclopentylaminopentanoyl-N,N-diethylamide; and
5-N-Benzoylaminopentanoyl-N,N-diethylamide.



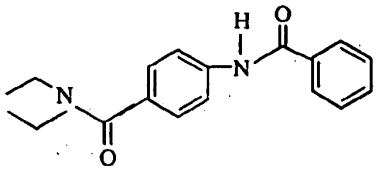
N-tert-butoxycarbonyloxyaminopentan-N,N-diethylamide



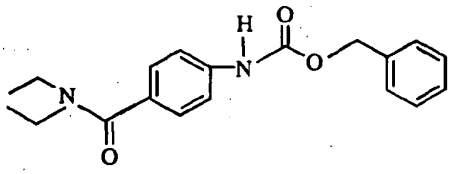
N-Benzoylalanyl-N,N-diethylamide



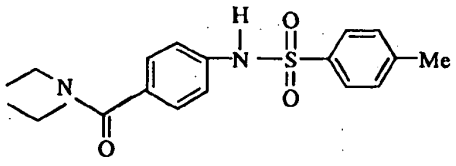
N-tert-butoxycarbonyloxyalanyl-N-methyl-N-phenylamide



4-Benzoylaminobenzoyl-N,N-diethylamid



4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide



4-Toluenesulfonylaminobenzoyl-N,N-diethyla
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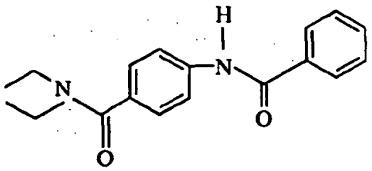
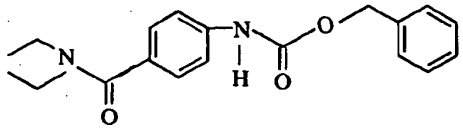
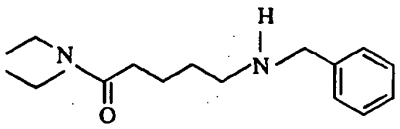


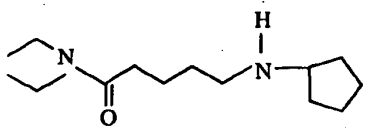
4-Benzoylaminobenzoyl-N,N-diethylamide



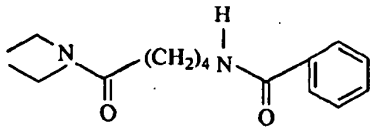
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide



5-N-Benzylaminopentanoyl-N,N-diethylamide



5-N-Cyclopentylaminopentanoyl-N,N-diethylamide



5-N-Benzoylaminopentanoyl-N,N-diethylamide

BOC = tert/butoxycarbonyl Me = CH_3

Examples of suitable indazoles include:

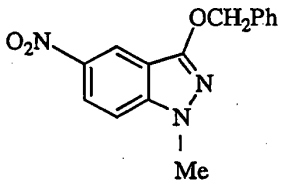
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-[(3-dimethylamino)propyloxy]-1*H*-indazole;
1-Benzyl-3-ethoxy-5-nitro-1*H*-indazole;
1-Methyl-3-ethoxy-5-benzoylamino-1*H*-indazole;
1-Methyl-3-ethoxy-5-benzylaminocarboxylamino-1*H*-indazole;
1-Methyl-3-tosyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-bromo-5-nitro-1*H*-indazole;
1-Methyl-3-benzyloxy-5-amino-1*H*-indazole;
1-Phenyl-3-hydroxy-5-nitro-1*H*-indazole;
1-Benzyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-[(3-dimethylamino)propyloxy]-5-amino-1*H*-indazole;
1-Benzyl-3-ethoxy-5-amino-1*H*-indazole;
Benzydamine 1-Methyl-3-[(dimethylamino)propyloxy-1*H*-indazole;
Isoniazid or 4-pyridinecarboxylic acid hydrazide;
1-Methyl-3-ethoxy-5-nitro-1*H*-Indazole; and
1-Methyl-3-ethoxy-5-amino-1*H*-Indazole.

Preferred indazoles are:

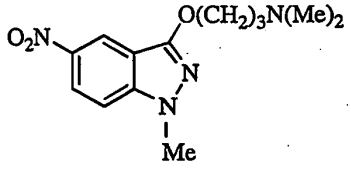
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Benzyl-3-ethoxy-5-nitro-1*H*-indazole;
1-Methyl-3-ethoxy-5-benzoylamino-1*H*-indazole;
1-Methyl-3-ethoxy-5-benzylaminocarboxylamino-1*H*-indazole;
1-Methyl-3-tosyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-benzyloxy-5-amino-1*H*-indazole;
1-Methyl-3-[(3-dimethylamino)propyloxy]-5-amino-1*H*-indazole;
1-Benzyl-3-ethoxy-5-amino-1*H*-indazole; and
Isoniazid or 4-pyridinecarboxylic acid hydrazide;

Most preferred indazoles are:

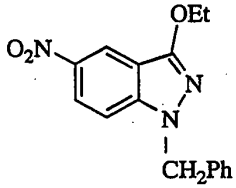
1-Methyl-3-[(3-dimethylamino)propyloxy]-1*H*-indazole;
1-Methyl-3-bromo-5-nitro-1*H*-indazole;
1-Phenyl-3-hydroxy-5-nitro-1*H*-indazole;
1-Benzyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-[(dimethylamino)propyloxy-1*H*-indazole;
1-Methyl-3-ethoxy-5-nitro-1*H*-Indazole; and
1-Methyl-3-ethoxy-5-amino-1*H*-Indazole.



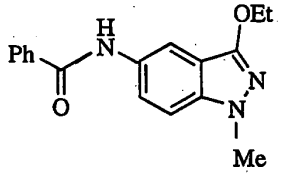
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole



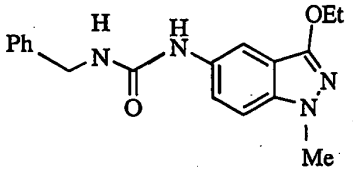
1-Methyl-3-[(3-dimethylamino)propyloxy]-1*H*-indazole



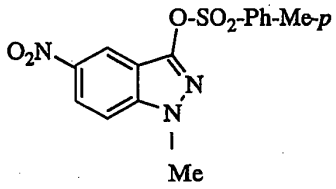
1-Benzyl-3-ethoxy-5-nitro-1*H*-indazole



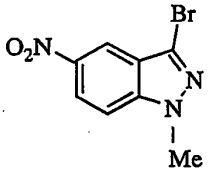
1-Methyl-3-ethoxy-5-benzoylamino-1*H*-indazole



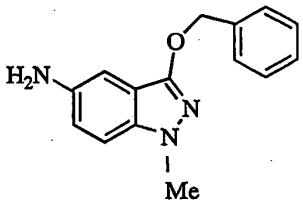
1-Methyl-3-ethoxy-5-benzylaminocarboxylamino-1*H*-indazole



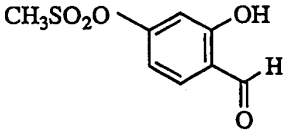
1-Methyl-3-tosyloxy-5-nitro-1*H*-indazole



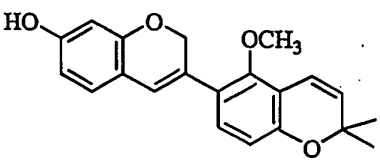
1-Methyl-3-bromo-5-nitro-1*H*-indazole



1-Methyl-3-benzyloxy-5-amino-1*H*-indazole

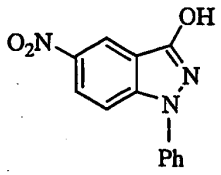


2-Hydroxy-4-mesyloxybenzaldehyde

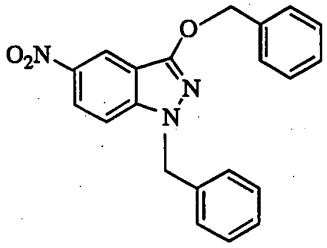


2'-O-Methyl-7-hydroxyisoglabrene

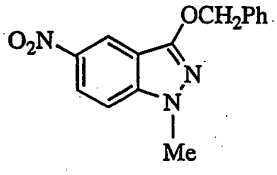
Et = CH_2CH_3 Me = CH_3 Ph = Phenyl



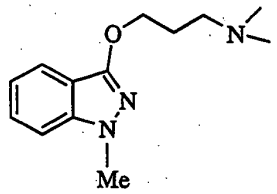
1-Phenyl-3-hydroxy-5-nitro-1*H*-indazole



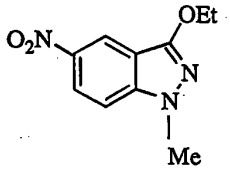
1-Benzyl-3-benzyloxy-5-nitro-1*H*-indazole



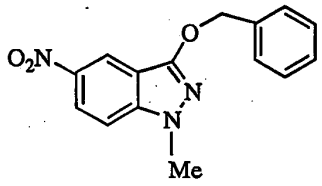
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole



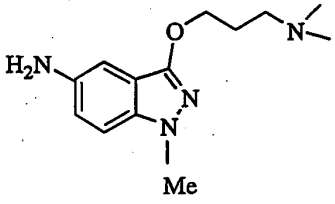
1-Methyl-3-[(dimethylamino)propyloxy-1*H*-indaz



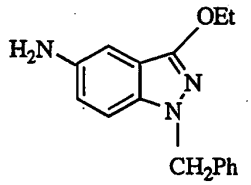
1-Methyl-3-ethoxy-5-nitro-1*H*-indazole



1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole



1-Methyl-3-[(3-dimethylamino)propyloxy]-5-amino-1*H*-indazole



1-Benzyl-3-ethoxy-5-amino-1*H*-indazol

Me = CH_3 Ph = Phenyl Et=CH_2CH_3

Examples of suitable isoglabrene analogs include:

4'-Methoxyisoflav-3-ene;

4'-Hydroxyisoflav-3-ene;

2'-Methoxyisoflav-3-ene;

7,2'-Dimethoxyisoflav-3-ene;

2,4'-Dimethoxyisoflav-3-ene;

7-Methoxy-3-[5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran6-yl]2-*H*-]-benzo-3-pyran;

2'*O*-Methylisoglabrene;
Isoglabrene;

2'-*O*-Methyl-3,4,3"4"-tetrahydroisoglabrene;

3"4"-Dihydroisoglabrene;

2'*O*-Methyl-7-demethyl-7-methanesulfonylisoglabrene;

7-Hydroxy-6-aceto-2,2-dimethylbenzo-1H-benzopyran;

5-Hydroxy-6-acetyl-2,2-dimethyl-2*H*-1-benzopyran;

5-Methoxy-6-[(2-hyroxymethyl-5-mesyloxy)-2-phenyloxy]aceto-2,2-dimethylbenzo-1*H*-benzopyran; and

2-hydroxy-4-mesyloxybenzylacohol.

Preferred isoglabrene analogs are:

4'-Hydroxyisoflav-3-ene;

7,2'-Dimethoxyisoflav-3-ene;

7-Methoxy-3-[5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran6-yl]2-*H*-]-benzo-3-pyran;

2'-*O*-Methyl-3,4,3"4"-tetrahydroisoglabrene;

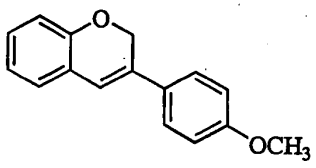
5-Hydroxy-6-aceryl-2,2dimethyl-2*H*-1-benzopyran;

2-hydroxy-4-mesyloxybenzylacohol; and

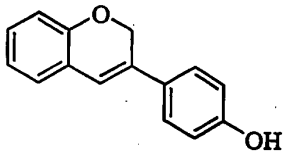
2'*O*-Methyl-7-demethyl-7-methanesulfonylisoglabrene.

Most preferred isoglabrene analogs are:

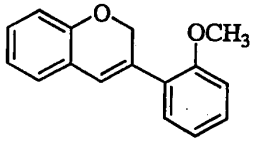
2'-Methoxyisoflav-3-ene;
2,4'-Dimethoxyisoflav-3-ene;
2'O-Methylisoglabrene;
Isoglabrene;
3"4"-Dihydroisoglabrene;
2'O-Methyl-7-demethyl-7-methanesulfonylisoglabrene;
7-Hydroxy-6-aceto-2,2-dimethylbenzo-1*H*-benzopyran; and
5-Methoxy-6-[(2-hyroxymethyl-5-mesyloxy)-2-phenyloxy]aceto-2,2-dimethylbenzo-1*H*-benzopyran.



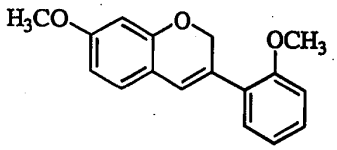
3"4"-Dihydroisoglabrene



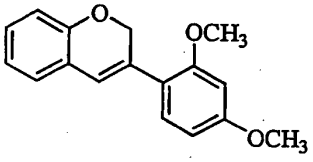
4'-Hydroxyisoflav-3-ene



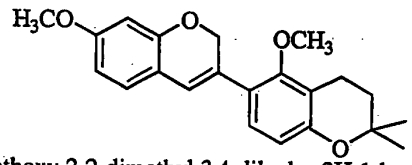
2'-Methoxyisoflav-3-ene



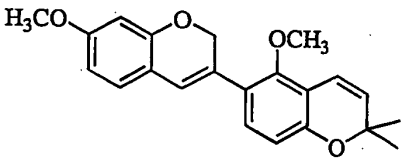
7,2'-Dimethoxyisoflav-3-ene



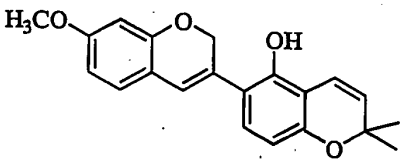
2,4'-Dimethoxyisoflav-3-ene



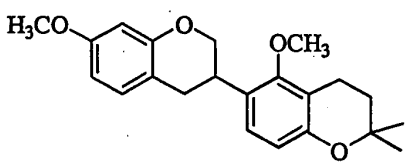
7-Methoxy-3-[5-methoxy-2,2-dimethyl-3,4-dihydro-2H-1-benzop
n6-yl]2-H-]-benzo-3-pyran



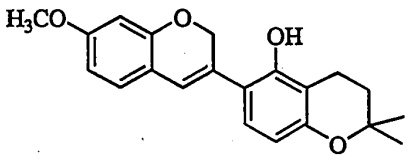
2'-*O*-Methylisoglabrene



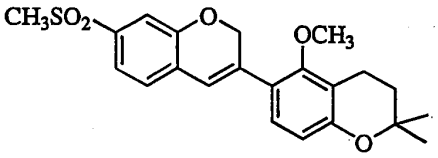
Isoglabrene



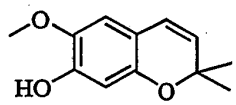
2'-O-Methyl-3,4,3"4"-tetrahydroisoglabrene



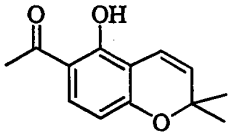
3"4"-Dihydroisoglabrene



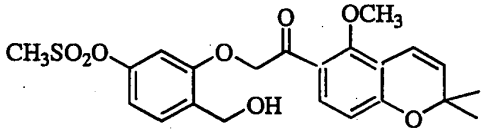
2-'O-Methyl-7-demethyl-7-methanesulfonylisoglabrene



7-Hydroxy-6-aceto-2,2-dimethylbenzo-1*H*-



5-Hydroxy-6-acertl-2,2-dimethyl-2*H*-1-benzopyran

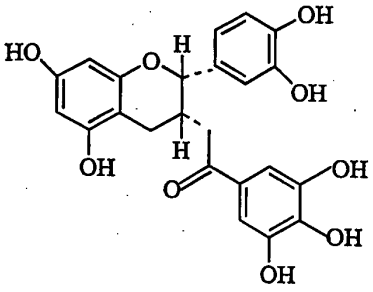


5-Methoxy-6-[(2-hyroxymethyl-5-mesyloxy)-2-aceto-2,2-dimethylbenzo-1*H*-benzopyran

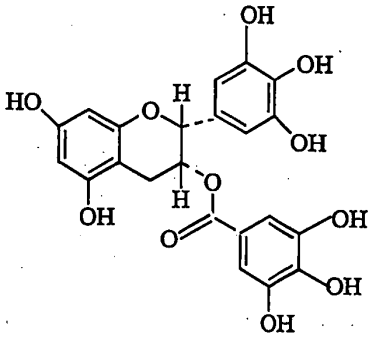
Suitable antioxidants include trolox, reduced glutathione, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), (-)-Epigallocatechin Gallate (EGCG), (-)-Gallocatechin Gallate (GCG), (-)-Epigallocatechin (EGC), (-)-Epicatechin Gallate (ECG) and (-)-Epicatechin (EC).

Preferred antioxidants are trolox, BHA, BHT, Gallocatechin, Gallate, Epigallocatechin, Epicatechin Gallate and Epicatechin.

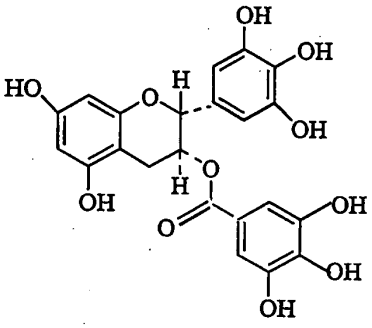
Most preferred antioxidants are reduced glutathione and Epigallocatechin Gallate.



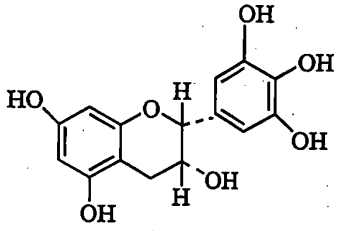
(-)-Epicatechin Gallate [(-)-ECG]



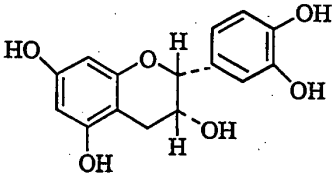
(-)-Epigallocatechin Gallate [(-)-ECCG]



(-)-Gallocatechin Gallate [GCG]



(-)-Epigallocatechin [ECG]



(-)-Epicatechin [(-)-EC]]

The combination of topical antimicrobials and the antimutagenic and/or antioxidant compounds requires that the antimutagenic and/or antioxidant compounds be used in an effective amount. As used herein, the term effective amount refers to that amount of antimutagenic and/or antioxidant compound that when combined with the topical antimicrobial agent decreases the amount of antimicrobial resistant colonies present in the antimicrobial by at least 20%. Thus, if a topical antimicrobial measures a antimicrobial resistant colony (RC) value of, for example, RC=20, the effective amount of antimutagenic and/or antioxidant compound added would need to reduce antimicrobial resistant colony value equal to RC=16 or lower. As will be shown later, not all antimutagenic and/or antioxidant compounds are effective in reducing antimicrobial resistant colonies in topical antimicrobials.

Why any effective amount of the antimutagenic and/or antiodoxidant compounds may be used and may widely vary, typical effective amounts when the compound is a pyrithione-containing compound will range from 0.1 to 25, preferably from 0.1 to 10, most preferably from 0.1 to 5 μg/ml on an individual basis. Other antimutagenic and/or antioxidant compounds may be used in effective amounts ranging from 1 to 50, preferably from 1 to 25, most preferably from 1 to 10 mg/ml on an individual basis.

The relative ratio of topical antimicrobial to antimutagenic and/or antioxidant useful in this invention ranges generally from 1: 0.1-50, preferably from 1: 0.1-10.0, more preferably from 1: 0.5-5.0, and most preferrably from 1:1, and vice versa based on weight.

The antimicrobial resistance blocking compositions of this invention may be used in a wide variety of applications. Such applications include antimicrobial skin care products, antimicrobial wound dressings, antimicrobial therapeutic gels, anticancer compositions, antimicrobial gloves, antimicrobial skin preparations, antimicrobial drapes, antimicrobial scrubs, antimicrobial gels, antimicrobial lotions, antimicrobial contact lenses, antimicrobial artificial skin grafts, antimicrobial gene delivery systems, antimicrobial polypeptide and antimicrobial household products to name a few.

The antimicrobial resistance blocking compositions of this invention may be included with other skin treatment additives. Examples of suitable additives include: skin protectants, anti-fungal compounds, surface active materials, cationic antimicrobials, natural oils, plant and marine derived bioactive and/or natural

products, phospholipids, liposomes, cyclodextrins and metal oxides (e.g., zinc, copper).

EXAMPLES

The present invention may be further understood by the following examples which are intended only to be illustrative and not restrictive of the present invention.

Materials and Methods

Laboratory based bacterial and yeast strains

The following bacterial strains were obtained from the American Type Culture Collection and were used in establishing antimicrobial and antibiotic sensitivity levels. ***Staphylococcus aureus*** ATCC 13709, ***Escherichia coli*** ATCC 9637, ***Salmonella choleraesuis*** ATCC 9184, ***Pseudomonas aeruginosa*** ATCC 27853, ***Klebsiella pneumoniae*** ATCC 10031, and ***Candida albicans*** ATCC 10231, ***Escherichia coli*** AG100 was obtained through the courtesy of Professor Stuart Levy of Tufts University, Boston, MA.

Chemicals

Triclosan (Irgasan DP300) was obtained from CIBA Specialty Chemical Corp.

To evaluate the degree of development of antimicrobial resistance, growth assays were performed using a selection of laboratory based bacteria in a 96 well ELISA plate format with varying concentrations of antiseptics and antibiotics in Oxoid Nutrient Broth No. 2 at 37°C. Readings were taken at 570 nm every 24 hours for 12 days using a Cambridge Technology, Inc., Plate Solver Ver. 4.00 and graphed as a function of growth vs. time. A second set of experiments used resistant strains of bacteria subcultured from survivors of the first experiment.

MIC and MBC assays

MIC and MBC assays were performed using various laboratory strains and multiple antibiotic resistant strains of bacteria collected from patients. The strains were grown in Oxoid Nutrient Broth No. 2 using a 96 well ELISA plate containing varying concentrations of test compounds with incubation for 15 hours at 37°C. After incubation the plates were examined using a Cambridge Technology, Inc., Plate Solver Ver. 4.00 at 570 nm. MIC-100 values were assigned from wells showing no absorbence. Following this, the media from wells showing no growth were streaked on Oxoid Nutrient Agar No. 2 plates and incubated for 15 hours at 37°C. MBC-100 values were assigned from plates showing no visible growth.

Antibiograms (Kirby-Bauer) agar diffusion method for determination of zones of inhibition.

Sterile blank disks (6 mm in diameter) were impregnated with 5 µl on 10 µg/ml solutions of a variety of antimicrobials. Incubation was at 37°C for four days on Oxoid Nutrient No. 2 Agar plates. The plates were read on the second day for determinations of the diameter of the inhibition zones and on the fifth day for resistance development data. Resistance was measured by estimating the percentage of the zone of inhibition that was covered by resistant colonies or, in other experiments, by counting the number of individual colonies that developed in the otherwise clear zone of inhibition.

Example 1 - Bacterial Resistance to Triclosan

When standard strains of bacteria were incubated for fourteen days in enriched media containing graded concentrations of triclosan ranging in two-fold increments from 0.25-256 µg/ml, ***Escherichia coli*** AG100 strain was controlled for 13 days by doses of 2 µg/ml or more, but breakthrough to resistance was seen at concentrations between the MIC and MBC values in the form of perceptible growth in 9 days with 1 µg/ml and at 5 days with 0.5 and 0.25 µg/ml of triclosan. ***Escherichia coli*** ATCC 9637 was controlled by doses of triclosan of 0.5 µg/ml and above but developed

resistance in 5 days at 0.25 μg/ml. With ***Klebsiella pneumoniae*** ATCC 10031, resistance breakthrough was seen at 9 days with 2 μg/ml, at 5 days with 1 μg/ml, at 4 days with 0.5 μg/ml and 0.25 μg/ml. ***Candida albicans*** ATCC 10231 developed resistance in 2 days at 2 μg/ml. S. aureus ATCC 13709 was controlled for 14 days by all concentrations employed, but ***Pseudomonas aeruginosa*** ATCC 27853 and ***Salmonella choleraesuis*** ATCC 98184 were unaffected by any of these doses (Table 1-1).

TABLE 1-1

Culture	Breakthrough time	Breakthrough level
S. aureus ATCC 13709	--	None
E. coli AG 100	9 days	1μg/ml
	5 days	0.5μg/ml*
	5 days	0.25μg/ml**
E. coli ATCC 9637	5 days	0.25μg/ml**
K. pneumoniae ATCC 10031	9 days	2μg/ml*
	5 days	1μg/ml*
	4 days	0.5μg/ml*
	4 days	0.25μg/ml
C. albicans ATCC 10231	2 days	2μg/ml
P. aeruginosa ATCC 27853	--	Not sensitive up to 256μg/ml
S. choleraesuis ATCC 9184	--	Not sensitive up to 256μg/ml

Table 1-1. Breakthrough levels to resistance by a series of laboratory microorganisms exposed to incremental doses of triclosan for 14 days, * indicates the growth did not reach the same level as control growth reached. ** indicates that growth reached the same level as the control growth reached but at the 11th day of incubation. Generally the controls reached maximal growth levels by day 2.

Colonies of ***E. coli*** ATCC 9637 and ***K. pneumoniae*** ATCC 10031 shown to be resistant to triclosan in this

manner failed to be inhibited by triclosan at any concentration between 0.25 and 256 μg/ml. On the other hand, triclosan resistant ***C. albicans*** ATCC 10231 was less resistant, showing growth in 5 days at 1 μg/ml, in 3 days at 0.5 μg/ml, and in 2 days at 0.25 μg/ml (Table 1-2). The ***C. albicans*** results suggest persistence rather than resistance.

TABLE 1-2

Culture	Breakthrough time	Breakthrough level
E. coli ATCC 9637 R at 0.25μg/ml	--	Insensitive from 0.25-256μg/ml
K. pneumoniae ATCC 10031 R2μg/ml	--	Insensitive from 0.25-256μg/ml
C. albicans ATCC 10231 R1μg/ml	5 days	1μg/ml
	3 days	0.5μg/ml
	2 days	0.25μg/ml

Table 1-2. Breakthrough levels to resistance by triclosan resistant bacteria when subsequently exposed to incremental doses of triclosan for 14 days. (R. figures indicate the concentration of triclosan from which these resistant colonies were cultured.)

The MIC-100 and MBC-100 values for triclosan against these ATCC strains and ***E. coli*** AG 100 were determined by agar dilution methods and these are listed in Table 1-3. The difference between these values is 2-8 fold indicating that bacteriocidal doses of triclosan can easily be reached through the use of comparatively

modest concentrations, except with ***Pseudomonas aeruginosa*** and ***Salmonella choleraesuis*** which are intrinsically highly resistant at the outset. Repetition of these measurements using strains deliberately made triclosan resistant showed that ***E. coli*** and ***K. pneumoniae*** became highly resistant, but the ***C. albicans*** strain was still sensitive to triclosan. These results are in general agreement with those in Tables 1-1 and 1-2.

TABLE 1-3

Culture	Triclosan		Triclosan-resistant	
	MIC	MBC	MIC	MBC
S. aureus ATCC 13709	0.25	1	--	--
E. coli ATCC 9637	0.25	0.5	>256	>256
E. coli AG 100	0.5	4	--	--
K. pneumoniae ATCC 10031	0.25	4	>256	>256
C. albicans ATCC 10231	2	4	2	4
P. aeuruginosa ATCC 27853	>256	>256	--	--
S. choleraesuis ATCC 9184	>256	>256	--	--

Table 1-3. A comparison of the MIC-100 and MBC-100 values (in µg/ml) for triclosan naïve and triclosan resistant strains of various bacteria.

Example 2 - Zinc Analogs

This example shows that when zinc pyrithione or sodium pyrithione are added to triclosan, the combination produces broad spectrum suppression of triclosan resistance, with the exception of ***Klebsiella pneumoniae.***

A variety of zinc containing analogs were examined and only zinc pyrithione gave useful MIC and MBC values when they were examined individually (Table 2-1). As noted in Table 2-1's footnote, zinc chloride, zinc sulfate, zinc acetate and zinc metal powder failed to inhibit. Sodium pyrithione gave somewhat less impressive MIC-100 values than zinc pyrithione and was ineffective at large doses in the MBC studies except, for ***S. aureus*** and ***C. albicans***.

TABLE 2-1

Culture	Zinc pyrithione		Sodium pyrithione	
	MIC-100	MBC-100	MIC-100	MBC-100
S. aureus ATCC 13709	0.5µg/ml	2µg/ml	1µg/ml	2µg/ml
E. coli AG100	0.5	4	4	>256
E. coli ATCC 9637	8	8	8	>256
K. pneumoniae ATCC 10031	2	4	8	>256
C. albicans ATCC 10231	0.5	2	0.25	4
P. aeruginosa ATCC 27853	32	32	64	>256
S. choleraesuis ATCC 9184	32	32	64	>256

Table 2-1. MIC-100 and MBC-100 levels for zinc pyrithione and sodium pyrithione against a variety of microorganisms. When tested under the same conditions, zinc chloride, zinc sulfate, zinc acetate and zinc metal powder were inactive below 128 µg/ml.

When zinc pyrithione and sodium pyrithione were added individually to disks also containing an equal concentration of triclosan, resistance development (as judged from the presence of no colonies within the zone of inhibition) was completely prevented in ***E. coli*** ATCC9637 and AG 100 but these compounds failed to

provide the same protective effect against ***K. pneumoniae*** ATCC 10031. No other zinc salt tested showed this effect (Table 2-2). Given its more bacteriocidial effect and somewhat broader spectrum, the combination of zinc pyrithione and triclosan is to be preferred.

TABLE 2-2

Culture	Triclosan	Zinc pyrithione	Triclosan + Zinc pyrithione
S. aureus ATCC 13709	54 mm (0)	15 mm (0)	57 mm (0)
E. coli AG100	31 (15)	18 (0)	35 (0)
E. coli ATCC 9637	28 (15)	14 (0)	28 (0)
K. pneumoniae ATCC 10031	35 (18)	14 (0)	35 (11)
C. *albicans* ATCC 10231	21 (0)	26 (0)	30 (0)
P. aeruginosa ATCC 27853	0 (0)	7 (0)	9 (0)
S. choleraesuis ATCC 9184	0 (0)	10 (0)	12 (0)

Culture	Triclosan	Sodium pyrithione	Triclosan + Sodium pyrithione
S. aureus ATCC 13709	49 mm (0)	29 mm (0)	48 mm (0)
E. coli AG100	31 (13)	33 (0)	31 (0)
E. coli ATCC 9637	28 (13)	26 (0)	31 (0)
K. pneumoniae ATCC 10031	35 (15)	29(0)	35 (15)
C. albicans ATCC 10231	24 (0)	42 (0)	42 (0)
P. aeruginosa ATCC 27853	0 (0)	0 (0)	0 (0)
S. choleraesuis ATCC 9184	0 (0)	0 (0)	0 (0)

Table 2-2. Effect of zinc and sodium pyrithione at 10 μg/ml on the zone sizes obtained for triclosan at the same concentration and on the development of resistant colonies in the resulting zones of inhibition. Cultures were incubated for 5 days at 37°c. The number of resistant colonies that developed are listed in the parentheses. Zinc acetate, zinc chloride, zinc sulfate and zinc metal powder were ineffective in producing zones or in reducing the number of resistant colonies that developed.

Interestingly, however, breakthrough to resistance could be observed when all of the cultures, except ***P. aeruginosa*** and ***S. choleraesuis***, were incubated for a long time (14 days) with zinc pyrithione (Table 2-3).

TABLE 2-3

Culture	Breakthrough time	Breakthrough level
S. aureus ATCC 13709	4 days	1μg/ml*
	4 days	0.5μg/ml*
E. coli AG 100	7 days	2μg/ml*
	3 days	1μg/ml*
	2 days	0.5μg/ml*
	2 days	0.25μg/ml*
E. coli ATCC 9637	11 days	4μg/ml*
	8 days	2μg/ml*
	5 days	1μg/ml*
	3 days	0.5μg/ml*
	2 days	0.25μg/ml*
K. pneumoniae ATCC 10031	4 days	2μg/ml*
C. albicans ATCC 10231	4 days	1μg/ml*
	2 days	0.5μg/ml*
P. aeruginosa ATCC 27853	--	Not sensitive up to 32μg/ml
S. choleraesuis ATCC 9184	--	Not sensitive up to 32μg/ml

Table 2-3. Breakthrough levels to resistance by a series of laboratory microorganisms exposed to incremental doses of zinc pyrithione for 14 days. * indicates that growth did not reach the same level as control growth reached. Generally the controls reached maximal growth levels by day 2.

Thus, in view of Examples 1 and 2, it is not difficult to demonstrate in the laboratory that certain common bacteria produce resistant or persistent colonies

when exposed to insufficient concentrations of triclosan. In our hands, in addition to ***E. coli*** AG100 for which resistance has been described by others, ATCC strains of ***E. coli, K. pneumoniae***, and ***C. albicans*** also produce such colonies as summarized in Tables 1-1 and 1-2. ***E. coli*** AG100 is significantly more prone to produce such colonies than ***E. coli*** ATCC strain 9637. ***K. pneumoniae*** ATC 10031 likewise produces resistant colonies over a ten-fold concentration range. These concentrations are significantly smaller than those used in triclosan preparations unless they are highly diluted. When picked and regrown in rich media, the triclosan-resistant colonies of ***E. coli*** and ***K. pneumoniae***, however, were found to be very resistant to triclosan (Table 1-2). The concentrations required to lyse the resistant colonies may well lie outside the concentrations one is advised to use. Interestingly, resistant colonies of ***C. albicans*** remained sensitive to triclosan under the same conditions. ***P. aeruginosa*** and ***S. choleraesuis*** were resistant from the outset, but ***S. aureus*** was sensitive to triclosan and did not develop resistant colonies in these experiments. It seems therefore, advisable to use triclosan preparations at significant concentrations so as to avoid the emergence of resistant strains.

In other experiments the MIC-100 and MBC-100 values were determined. Triclosan-resistant ***E. coli*** and ***K.***

pneumoniae were 1000-fold less sensitive to triclosan whereas triclosan-resistant ***C. albicans*** retained its sensitivity values (Table 1-3).

These methods should be useful in detecting other agents that have similar useful activity. The data in Table 2-1 show that zinc and sodium pyrithione, an antiinfective agent frequently used in cosmetics, are active against both the Gram positive and Gram negative bacteria used in this study. The use of the pyrithione salts along with triclosan resulted in the elimination of resistant colony development, once again with the exception of ***K. pneumoniae*** (Table 2-2). Since zinc pyrithione is bacteriocidal at much smaller doses than the sodium salt, it should provide a smaller opportunity for resistant colony development and is the preferred salt to use.

Example 3 - Coumarins

This example summarizes the effectiveness of coumarins on the prevention of Triclosan (IRGASAN DP300) antimicrobial resistance development in ***E. coli*** ATCC 9637.

Table 3A shows the effect of Galbanic acid analogs on the prevention of Triclosan (IRGASAN DP300) antimicrobial resistance development in ***E. coli*** ATCC 9637. In this case, 5µl of 10mg/ml solution was

embedded for each compound on a 6mm sterile disk.

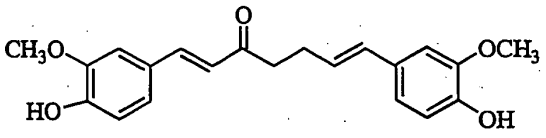
The diameter of the zone of inhibition was measured in mm after incubation for 5 days at 37°C. The number of resistant colonies within the zone of inhibition was recorded down as RC values.

TABLE 3A

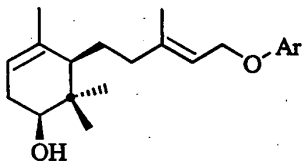
Strain ***E. coli*** ATCC 9637

Compound	Zone of Inhibition
IRGASAN DP300	27 mm (RC=12)
Galbanic Acid	24 mm (RC=7)
[(2'2,6-Trimethyl-2-oxo-bicyclo)-2,2,1-heptyl]-1-ß-3-methyl-pent-2-enyl-7-oxycoumarin	23 mm (RC=0)
4'4'Dihydroxy-3,3'Dimethoxy-Dicinnamyl ester	28 mm (RC=10)
[(2'2-Dimethyl-3-ß-hydroxy-6-methylene)-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycoumarin	26 mm (RC=1)
[(2'2-Dimethyl-3-ß-hydroxy-6-methyl)-5-dehydro-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycoumarin	26 mm (RC=10)
[(2'2-Dimethyl-3-ß-hydroxy-6-ß-hydroxy-6-methyl-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycourmarin	26 mm (RC=12)

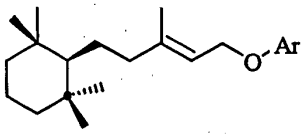
Table 3B shows the effect of coumarin analogs on the prevention of Triclosan (IRGASAN DP300) antimicrobial resistance development in ***E. coli*** ATCC 9637. In this case, 5µl of 10mg/ml solution was embedded for each compound on a 6mm sterile disk. The diameter of the zone of inhibition was measured in mm after incubation for 5 days at 37°C. The number of resistant colonies within the zone of inhibition was recorded down as RC values.



4'4'Dihydroxy-3,3'Dimethoxy-Dicinnamyl ester



[(2'2-Dimethyl-3-ß-hydroxy-6-methyl-5-deh
ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycc



[(2'2-Dimethyl-3-ß-hydroxy-6-ß-hydroxy-6-methyl-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycourn

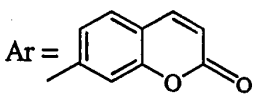


TABLE 3B

Strain ***E. coli*** ATCC 9637

Compound	Zone of Inhibition
IRGASAN DP300	28 mm (RC=12)
7-Hydroxycoumarin	27 mm (RC=11)
7-1,1-Dimethylprop-2-ynyloxycoumarin	26 mm (RC=11)
8-(2'2'-Dimethyl-1'H-pyran-7-yl)coumarin	22 mm (RC=7)
7-Cyclohexylmethoxycoumarin	28 mm (RC=5)
7-[3-Methyl-1butenyloxy]coumarin	28 mm (RC=11)
7-Methoxycoumarin	27 mm (RC=11)

As can be seen from Tables 3A and 3B, the results show that only the combinations of triclosan with:

8-(2'2'-Dimethyl-1'H-pyran-7-yl)coumarin;

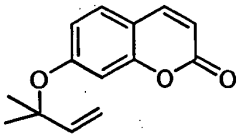
[(2'2-Dimethyl-3-ß-hydroxy-6-methylene)-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycoumarin;

[(2'2,6-Trimethyl-2-oxo-bicyclo)-2,2,1-heptyl]1-ß-3-methyl-pent-2-enyl-7-oxycoumarin;

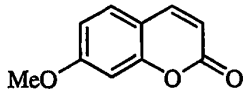
7-Cyclohexylmethoxycoumarin; and

Galbanic Acid

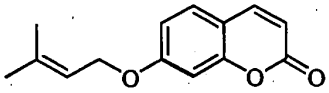
provided compositions that were effective to reduce antimicrobial blocking resistance by at least 20 percent.



7-1,1-Dimethylprop-2-ynyloxycoumarin



7-Methoxycoumarin



7-[3-Methyl-1butenyloxy]coumarin

Example 4 - Pseudopeptides

This example summarizes the effectiveness of pseudopeptides on the prevention of Triclosan (IRGASAN DP300) antimicrobial resistance development in ***E.coli*** ATCC 9637. In this case, 5μl of 10mg/ml solution was embedded for each compound on a 6mm sterile disk. The diameter of the zone of inhibition was measured in mm after incubation for 5 days at 37°C. The number of resistant colonies within the zone of inhibition was recorded down as RC values. The results are shown Table 4-1.

TABLE 4-1

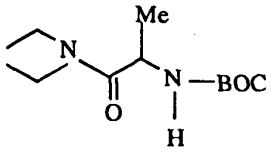
Strain ***E. coli*** ATCC 9637

Compound	Zone of Inhibition
IRGASAN DP300	25 mm (RC=12)
N-tert-butoxycarbonyloxyaminopentan-N,N-diethylamide	23 mm (RC=8)
N-[4-Toluenesulfonyl]alanyl-N,N-diethylamide	26 mm (RC=12)
N-Benzoylalanyl-N,N-diethylamide	25 mm (RC=6)
N--benzyloxycarbonylalanyl-N,N-diethylamide	25 mm (RC=10)
N-tert-Butoxycarbonyloxyalanyl-N,N-diethylamide	25 mm (RC=5)
N-tert-butoxycarbonyloxyalanyl-N-methyl-N-phenylamide	24 mm (RC=9)
N-tert-Butoxycarbonyloxyalanyl-N,N-morphilinoamide	27 mm (RC=9)
4-Benzoylaminobenzoyl-N,N-diethylamide	24 mm (RC=7)
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide	23 mm (RC=5)
4-Toluenesulfonylaminobenzoyl-N,N-diethylamide	23 mm (RC=6)
4-Benzoylaminobenzoyl-N,N-diethylamide	21 mm (RC=6)
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide	22 mm (RC=4)
5-N-Benzylaminopentanoyl-N,N-diethylamide	25 mm (RC=5)
5-N-Cyclopentylaminopentanoyl-N,N-diethylamide	26 mm (RC=4)
5-N-toluensefulfonylaminopentanoyl-N,N-diethylamide	25 mm (RC=10)
5-N-Benzoylaminopentanoyl-N,N-diethylamide	26 mm (RC=3)
N-Benzylalanyl-N,N-diethylamide	25 mm (RC=13)

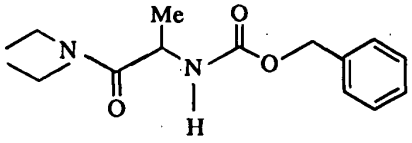
As can be seen from Table 4-1, the results show that only the combinations of triclosan with:

N-tert-butoxycarbonyloxyaminopentan-N,N-diethylamide;
N-Benzoylalanyl-N,N-diethylamide;
N-tert-Butoxycarbonyloxyalanyl-N,N-diethylamide;
N-tert-Butoxycarbonyloxyalanyl-N,N-morphilinoamide;
N-tert-butoxycarbonyloxyalanyl-N-methyl-N-phenylamide;
4-Benzoylaminobenzoyl-N,N-diethylamide;
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide;
4-Toluenesulfonylaminobenzoyl-N,N-diethylamide;
4-Benzoylaminobenzoyl-N,N-diethylamide;
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide;
5-N-Benzylaminopentanoyl-N,N-diethylamide;
5-N-Cyclopentylaminopentanoyl-N,N-diethylamide; and
5-N-Benzoylaminopentanoyl-N,N-diethylamide

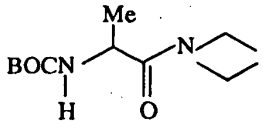
provided compositions that were effective to reduce antimicrobial blocking resistance by at least 20 percent.



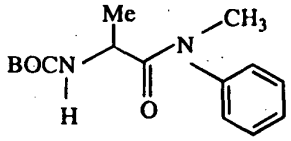
N-tert-butoxycarbonyloxyaminopentan-N,N-diethylamide



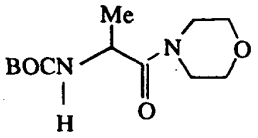
N--benzyloxycarbonylalanyl-N,N-diethylamide



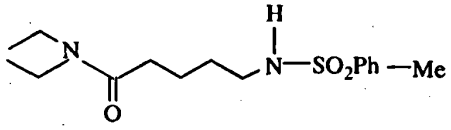
N-tert-Butoxycarbonyloxyalanyl-N,N-diethylamide



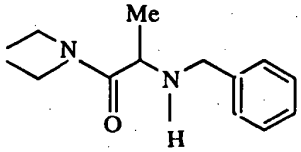
N-tert-butoxycarbonyloxyalanyl-N-methyl-N-phenylamide



N-tert-Butoxycarbonyloxyalanyl-N,N-morphilinoamide



5-N-toluensefulfonylaminopentanoyl-N,N-diethylamide



N-Benzylalanyl-N,N-diethylamide

BOC = tert/butoxycarbonyl Me = CH_3

Example 5 - Indazole Analogs

This example summaries the effectiveness of Indazole analogs on the prevention of Triclosan (IRGASAN DP300) antimicrobial resistance development in ***E. coli*** ATCC 9637. In this case, 5μl of 10mg/ml solution was embedded for each compound on a 6 mm sterile disk. The diameter of the zone of inhibition was measured in mm after incubation for 5 days at 37°C. The number of resistant colonies within the zone of inhibition was recorded down as RC values.

TABLE 5-1

Strain ***E. coli*** ATCC 9637

Compound	Zone of Inhibition
IRGASAN DP300	28 mm (RC=12)
1-Methyl-3-ethoxy-5-benzylaminocarboxylamino-1*H*-indazole	27 mm (RC=8)
3-ethoxy-5-N-Tolune-sulfonyl-1H-indazole	25 mm (RC=11)
1-benzyl-3-hydroxy-5-nitro-1H-indazole	28 mm (RC=11)
1-Benzyl-3-ethoxy-5-amino-1*H*-indazole	27 mm (RC=6)
1-methyl-3-hydroxy-5-nitro-1*H*-indazole	29 mm (RC=11)
1-phenyl-3-hydroxy-5-nitro-1*H*-indazole	28 mm (RC=15)
Benzydamine/1-methyl-3-[(3-dimethylamino)propoxy]-1*H*-indazole	25 mm (RC=4)
Isoniazid or 4-pyridinecarboxylic acid hydrazide	28 mm (RC=9)
1-Methyl-3-bromo-5-nitro-1*H*-indazole	28 mm (RC=4)
1-methyl-3-ethoxy-5-aminobenzyl-1H-indazole	26 mm (RC=10)
1-methyl-3-ethoxy-5-nitro-1H-Indazole	28 mm (RC=2)
1-Methyl-3-tosyloxy-5-nitro-1*H*-indazole	26 mm (RC=7)
1-Benzyl-3-ethoxy-5-nitro-1*H*-indazole	26 mm (RC=6)
1-methyl-3-hydroxy-5-amino-1*H*-indazole hydrochloride	28 mm (RC=16)
1-Phenyl-3-hydroxy-5-nitro-1*H*-indazole	24 mm (RC=2)
1-Benzyl-3-benzyloxy-5-nitro-1*H*-indazole	27 mm (RC=4)
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole	27 mm (RC=9)
1-Methyl-3-ethoxy-5-benzoylamino-1*H*-indazole	26 mm (RC=6)

1-methyl-3-ethoxy-5-1H-inadazole	28 mm (RC=15)
1-methyl-3-ethoxy-5-amino-1*H*-Indazole	28 mm (RC=5)
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole	25 mm (RC=3)
1-Methyl-3-benzyloxy-5-amino-1*H*-indazole	26 mm (RC=6)
1-Methyl-3-[(3-dimethylamino)propyloxy]-5-amino-1*H*-indazole	27 mm (RC=9)
1-Methyl-3-[(3-dimethylamino)propyloxy]-1*H*-indazole	26 mm (RC=0)

As can be seen from Table 5-1, the results show that only the combinations of triclosan with:

1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole;

1-Methyl-3-[(3-dimethylamino)propyloxy]-1*H*-indazole;

1-Benzyl-3-ethoxy-5-nitro-1*H*-indazole;

1-Methyl-3-ethoxy-5-benzoylamino-1*H*-indazole;

1-Methyl-3-ethoxy-5-benzylaminocarboxylamino-1*H*-indazole;

1-Methyl-3-tosyloxy-5-nitro-1*H*-indazole;

1-Methyl-3-bromo-5-nitro-1*H*-indazole;

1-Methyl-3-benzyloxy-5-amino-1*H*-indazole;

1-Phenyl-3-hydroxy-5-nitro-1*H*-indazole;

1-Benzyl-3-benzyloxy-5-nitro-1*H*-indazole;

1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole;

1-Methyl-3-[(3-dimethylamino)propyloxy]-5-amino-1*H*-indazole;

1-Benzyl-3-ethoxy-5-amino-1*H*-indazole;

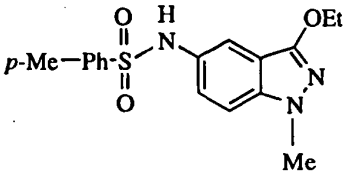
Benzydamine 1-Methyl-3-[(dimethylamino)propyloxy-1*H*-indazole;

Isoniazid or 4-pyridinecarboxylic acid hydrazide;

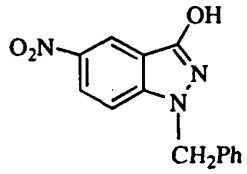
1-methyl-3-ethoxy-5-nitro-1*H*-Indazole; and

1-methyl-3-ethoxy-5-amino-1*H*-Indazole

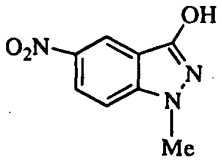
provided compositions that were effective to reduce antimicrobial blocking resistance by at least 20 percent.



3-ethoxy-5-N-Toluene-sulfonyl-1*H*-indazole



1-benzyl-3-hydroxy-5-nitro-1*H*-indazole



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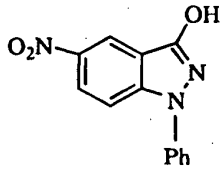
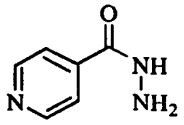


1-phenyl-3-hydroxy-5-nitro-1*H*-indazole



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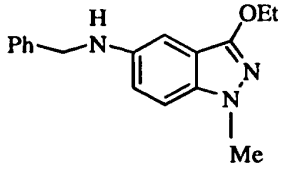
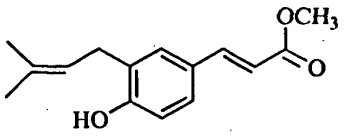
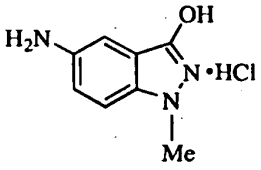


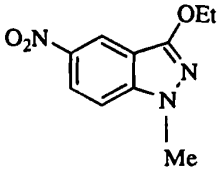
1-methyl-3-ethoxy-5-aminobenzyl-1*H*-indazole



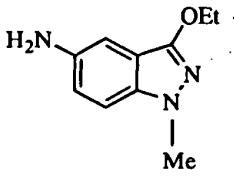
3-prenyl-4-hydroxy-cinnamyl methyl ester



1-methyl-3-hydroxy-5-amino-1*H*-indazole hydrochloride



1-methyl-3-ethoxy-5-1*H*-inadazole



1-methyl-3-ethoxy-5-amino-1H-Indazole

Et = CH_2CH_3 Me = CH_3 Ph = Phenyl

Example 6- Isoglabrene Analogs

This example summarizes the effectiveness of flavanoids and isoflavanoids including isoflavenes, isoflavanes, isoflavanones and isoglabrene analogs on the prevention of Triclosan (IRGASAN DP300) antimicrobial resistance development in ***E. coli*** ATCC 9637. In this case, 5 μl of 10mg/ml solution was embedded for each compound on a 6 mm sterile disk. The diameter of the zone of inhibition was measured in mm after incubation for 5 days at 37°C. The number of resistant colonies within the zone of inhibition was recorded down as RC values.

TABLE 6-1

Strain ***E. coli*** ATCC 9637

Compound	Zone of Inhibition
IRGASAN DP300	25 mm (RC=11)
2'4',7-Trimethoxyisoflav-3-ene	24 mm (RC=10)
Glycerizha-glabra ethanol extract	27 mm (RC=11)
SRM-I-195/2'*O*-Methyl-7-demethyl-7-methanesulfonylisoglabrene	23 mm (RC=2)
2'-O-Methyl-7-demthyl-7-hydroxyisoglabrene	24 mm (RC=9)
5-Methoxy-6-[(2-hyroxymethyl-5-mesyloxy)-2-phenyloxy]aceto-2,2-dimethylbenzo-1*H*-benzopyran	25 mm (RC=3)
2'0-Methyl-7-dimethyl-7-methanesulfonylisoglabrene	25 mm (RC=8)
2-hydroxy-4-mesyloxybenzylacohol	27 mm (RC=6)
5-Hydroxy-6-acet mmyl-2,2dimethyl-2*H*-1-benzopyran	23 mm (RC=7)
7-Hydroxy-6-aceto-2,2-dimethylbenzo-1H-benzopyran	24 mm (RC=5)
2-Hydroxy-4-mesyloxybenzaldehyde	27 mm (RC=18)
5-Hydroxy-6-acetyl-2,2-dimethyl-2*H*-1-benzopyran	26 mm (RC=0)
2'*O*-Methylisoglabrene	25 mm (RC=5)
2'4',-Dihydroxyisoflav-3-ene	26 mm (RC=13)
6"*O*-Methylisoglabrene	23 mm (RC=13)
4'-Hydroxyisoflav-3-ene	27 mm (RC=8)
Isoflav-3-ene	25 mm (RC=10)

4'7-Dihydroxyisoflav-3-ene	28 mm (RC=14)
Isoglabrene	25 mm (RC=2)
2'-*O*-Methyl-3,4,3"4"-tetrahydroisoglabrene	23 mm (RC=8)
7-Methoxy-3[5.methoxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran6-yl]2-*H*-]-benzo-3-pyran	24 mm (RC=8)
4'7-Dimethoxyisoflav-3-ene	27 mm (RC=13)
4'-Methoxyisoflav-3-ene	25 mm (RC=12)
2,4'-Dimethoxyisoflav-3-ene	22 mm (RC=2)
2'-Methoxyisoflav-3-ene	23 mm (RC=1)
7,2'-Dimethoxyisoflav-3-ene	24 mm (RC=7)

As can be seen in Table 6-1, the results show that only the combinations of triclosan with:

4'-Methoxyisoflav-3-ene;

4'-Hydroxyisoflav-3-ene;

2'-Methoxyisoflav-3-ene;

7,2'-Dimethoxyisoflav-3-ene;

2,4'-Dimethoxyisoflav-3-ene;

7-Methoxy-3-[5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran6-yl]2-*H*-]-benzo-3-pyran;

2'*O*-Methylisoglabrene;

Isoglabrene;

2'-*O*-Methyl-3,4,3"4"-tetrahydroisoglabrene;

3"4"-Dihydroisoglabrene;

2'*O*-Methyl-7-demethyl-7-methanesulfonylisoglabrene;

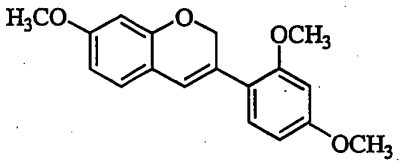
7-Hydroxy-6-aceto-2,2-dimethylbenzo-1H-benzopyran;

5-Hydroxy-6-acetyl-2,2dimethyl-2*H*-1-benzopyran;

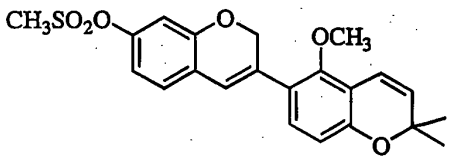
5-Methoxy-6-[(2-hyroxymethyl-5-mesyloxy)-2-phenyloxy]aceto-2,2-dimethylbenzo-1*H*-benzopyran; and

2-hydroxy-4-mesyloxybenzylacohol

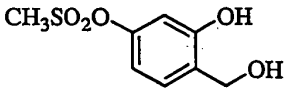
provided compositions that were effective to reduce antimicrobial blocking resistance by at least 20 percent.



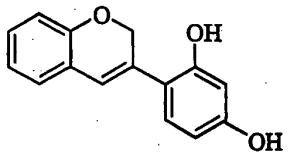
2'4',7-Trimethoxyisoflav-3-ene



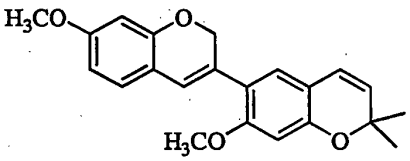
2'O-Methyl-7-demethyl-7-methanesulfonylisoglabrene



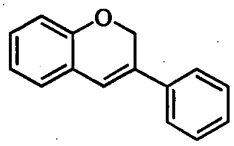
2-hydroxy-4-mesyloxybenzylacohol



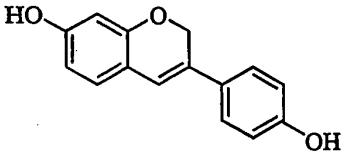
2'4',-Dihydroxyisoflav-3-ene



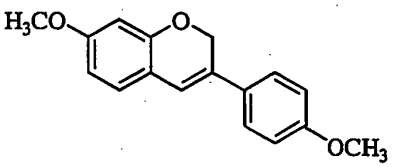
6"*O*-Methylisoglabrene



Isoflav-3-ene



4'7-Dihydroxyisoflav-3-ene



4'7-Dimethoxyisoflav-3-ene

Example 7- Antioxidants

This example summarizes the effectiveness of antioxidants on the prevention of Triclosan (IRGASAN DP300) antimicrobial resistance development in ***E. coli*** ATCC 9637. In this case, 5 µl of 10mg/ml solution was embedded for each compound on a 6 mm sterile disk. The diameter of the zone of inhibition was measured in mm after incubation for 5 days at 37°C. The number of resistant colonies within the zone of inhibition was recorded down as RC values.

TABLE 7

Strain ***E. coli*** ATCC 9637

Compound No.	Zone of Inhibition
IRGASAN DP300	28 mm (RC=12)
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox™)	27 mm (RC=9)
L-Ascorbic Acid	28 mm (RC=15)
Quercetin dihydrate	28 mm (RC=15)
Selenium	28 mm (RC=15)
Glutathione reduced	28 mm (RC=5)
Ellagic acid dihydrate	28 mm (RC=11)
BHT: butylated hydroxytoluene	28 mm (RC=8)
BHA: butylated hydroxyanisole	27 mm (RC=9)
N-acetyl-L-cysteine	27 mm (RC=12)
Curcumin	25 mm (RC=14)
EGCG: (-)-Epigallocatechin Gallate	27 mm (RC=5)
GCG: (-)-Gallocatechin Gallate	27 mm (RC=7)
EGC: (-)-Epicatechin Gallate	27 mm (RC=6)
ECG: (-)-Epicatechin Gallate	27 mm (RC=6)
EC: (-)-Epicatechin	27 mm (RC=6)

As can be seen from Table 7, the results show that only the combinations of triclosan with: 6-hydroxy-

2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX™), reduced glutathione, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), (-)-Epigallocatechin Gallate (EGCG), (-)-Gallocatechin Gallate (GCG), (-)-Epigallocatechin (EGC), (-)-Epicatechin Gallate (ECG) and (-)-Epicatechin (EC) provided compositions that were effective to reduce antimicrobial blocking resistance by at least 20 percent.

It should be understood that the foregoing disclosure and description of the present invention are illustrative and explanatory thereof and various changes in the size, shape and materials as well as in the description of the preferred embodiment may be made without departing from the spirit of the invention.

What is claimed is:

1. A topical antimicrobial composition comprising:

(c) a topical antimicrobial agent; and

(d) an antimicrobial resistance blocking effective amount of at least one antimutagenic and/or antioxidant agent.

2. The composition of claim 1, wherein the antimicrobial agent is selected from the group consisting of nisin, bis- diguanides, chlorhexidine gluconate, chlorhexidine digluconate, chlorhexidine diacetate, chlorhexidine dihydrochloride, polyhexamethylene biguanide, benzalkonium chloride, benzethonium chloride, methylbenzethonium chloride, cetyl pyridinium chloride, triclosan, triclocarban, tribromsalani, amyltricresols, parachlorometaxylenol, phenol, silver, iodine, nonylphenoxypoly (ethyleneoxy) ethanoliodine, poloxameriodine complex, undecoylium chloride, iodine complex, bisquaternary ammonium compounds, polymeric quaternary ammonium compounds, alcohols, cationic peptides, organometallic antiseptics, alkyl pyridinium salts, essential oils, and combinations and derivatives thereof.

3. The composition of claim 2, wherein the antimicrobial agent is triclosan.

4. The composition of claim 2, wherein the antimicrobial agent is chlorhexidine.

5. The composition of claim 2, wherein the antimicrobial agent is benzalkonium chloride.

6. The composition of claim 2, wherein the antimicrobial agent is benzethonium chloride.

7. The composition of claim 2, wherein the antimicrobial agent is triclocarban.

8. The composition of claim 2, wherein the antimicrobial agent is silver.

9. The composition of claim 2, wherein the antimicrobial agent is an alcohol selected from the group consisting of ethanol, propanol, butanol, pentanol, 2-methyl-1-butanol, hexanol, 2-methyl-1-pentanol, 3-methyl-1- pentanol, 2-ethyl-1-butanol, 3,5,5-trimethyl-1-hexanol, heptanol, octanol, isooctyl alcohol, decanol, dodecanol, tridecanol, tetradecanol and mixtures thereof.

10. The composition of claim 2, wherein the antimutagenic or antioxidant agent is selected from the group consisting of substituted and unsubstituted pyrithione-containing compounds; coumarins;

pseudopeptides; indazoles; antioxidants; flavanoids; isoflavanoids including isoflavenes, isoflavanes, isoflavanones and isoglabrene analogs; and mixtures thereof.

11. The composition of claim 10, wherein the substituted and unsubstituted pyrithione-containing compounds are monovalent or divalent metallic salts of pyrithione.

12. The composition of claim 11, wherein the pyrithione-containing compound is zinc or sodium pyrithione.

13. The composition of claim 12, wherein the pyrithione-containing compound is zinc pyrithione.

14. The composition of claim 12 or 13 wherein the antimicrobial agent is triclosan.

15. The composition of claim 10, wherein the coumarins are 8-(2'2'-Dimethyl-1'H-pyran-7-yl)coumarin ; [(2'2-Dimethyl-3-ß-hydroxy-6-methylene)-1-ß-cyclohexyl]-3-methyl-pent-2-enyl-7-oxycoumarin; [(2'2,6-Trimethyl-2-oxo-bicyclo)-2,2,1-heptyl]-1-ß-3-methyl-pent-2-enyl-7-oxycoumarin; 7-Cyclohexylmethoxycoumarin ; and galbanic acid.

16. The composition of claim 15, wherein the antimicrobial agent is triclosan.

17. The composition of claim 10, wherein the pseudopeptides are:

N-Tert-butoxycarbonyloxyaminopentan-N,N-diethylamide;
N-Benzoylalanyl-N,N-diethylamide;
N-tert-Butoxycarbonyloxyalanyl-N,N-diethylamide;
N-tert-Butoxycarbonyloxyalanyl-N,N-morphilinoamide;
N-Tert-butoxycarbonyloxyalanyl-N-methyl-N-phenylamide;
4-Benzoylaminobenzoyl-N,N-diethylamide;
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide;
4-Tol+uenesulfonylaminobenzoyl-N,N-diethylamide;
4-Benzoylaminobenzoyl-N,N-diethylamide;
4-Benzyloxycarbonyloxybenzoyl-N,N-diethylamide;
5-N-Benzylaminopentanoyl-N,N-diethlamide;
5-N-Cyclopentylaminopentanoyl-N,N-diethylamide; and
5-N-Benzoylaminopentanoyl-N,N-diethylamide.

18. The composition of claim 17, wherein the antimicrobial agent is triclosan.

19. The composition of claim 10, wherein the indazoles are:

1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-[(3-dimethylamino)propyloxy]-1*H*-indazole;
1-Benzyl-3-ethoxy-5-nitro-1*H*-indazole;
1-Methyl-3-ethoxy-5-benzoylamino-1*H*-indazole;
1-Methyl-3-ethoxy-5-benzylaminocarboxylamino-1*H*-indazole;

1-Methyl-3-tosyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-bromo-5-nitro-1*H*-indazole;
1-Methyl-3-benzyloxy-5-amino-1*H*-indazole;
1-Phenyl-3-hydroxy-5-nitro-1*H*-indazole;
1-Benzyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-benzyloxy-5-nitro-1*H*-indazole;
1-Methyl-3-[(3-dimethylamino)propyloxy]-5-amino-1*H*-indazole;
1-Benzyl-3-ethoxy-5-amino-1*H*-indazole;
1-Methyl-3-[(dimethylamino)propyloxy-1*H*-indazole;
Isoniazid or 4-pyridinecarboxylic acid hydrazide;
1-methyl-3-ethoxy-5-nitro-1*H*-Indazole; and
1-methyl-3-ethoxy-5-amino-1*H*-Indazole.

20. The composition of claim 19, wherein the antimicrobial agent is triclosan.

21. The composition of claim 10, wherein the flavanoids and isoflavanoids.

22. The composition of claim 21, wherein the isoflavonoids are of substituted and unsubstituted isoflavanes, isoflavenes, isoflavones and isoglabrene analogs.

23. The composition of claim 21, wherein the isoglabrene analogs are:
4'-Methoxyisoflav-3-ene;
4'-Hydroxyisoflav-3-ene;
2'-Methoxyisoflav-3-ene;
7,2'-Dimethoxyisoflav-3-ene;

2,4'-Dimethoxyisoflav-3-ene;
7-Methoxy-3[5-methoxy-2,2-dimethyl-3,4-dihydro-2*H*-1-benzopyran6-yl]2-*H*-]-benzo-3-pyran;
2'*O*-Methylisoglabrene;
Isoglabrene;
2'-*O*-Methyl-3,4,3"4"-tetrahydroisoglabrene;
3"4"-Dihydroisoglabrene;
2'*O*-Methyl-7-demethyl-7-methanesulfonylisoglabrene;
7-Hydroxy-6-aceto-2,2-dimethylbenzo-1H-benzopyran;
5-Hydroxy-6-acetyl-2,2-dimethyl-2*H*-1-benzopyran;
5-Methoxy-6-[(2-hyroxymethyl-5-mesyloxy)-2-phenyloxy]aceto-2,2-dimethylbenzo-1*H*-benzopyran; and
2-hydroxy-4-mesyloxybenzylacohol.

24. The composition of claim 23, wherein the antimicrobial agent is triclosan.

25. The composition of claim 10, wherein the antioxidants are 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX™), reduced glutathione, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), (-)-Epigallocatechin Gallate (EGCG), (-)-Gallocatechin Gallate (GCG), (-)-Epigallocatechin (EGC), (-)-Epicatechin Gallate (ECG) and (-)-Epicatechin (EC) .

26. The composition of claim 25, wherein the antimicrobial agent is triclosan.

27. The composition of claims 1-10, wherein the relative ratio of topical antimicrobial agent to

antimutagenic and/or antioxidant agent is in the range of from about 1 to about 0.1-50 based on weight and vice versa.

28. The composition of claims 1-10, wherein the antimicrobial agent and the antimutagenic and/or antioxidant agent are each present in a concentration amount ranging from about 0.0001 to about 50 mg/ml.

29. The composition of claim 28 where the antimutagenic and/or antioxidant agent is a pyrithione-containing compound present in a concentration amount ranging from about 0.1 to 5 μg/ml.

30. A method for inhibiting bacterial resistance comprising:

(a) incorporating an antimicrobial resistance blocking effective amount of at least one antimutagenic and/or antioxidant agent into a topical antimicrobial agent; and

(b) applying the composition of (a) to a surface containing bacteria.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 01/30303

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K45/06 A61P31/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 27792 A (NOVAPHARM RESEARCH) 10 June 1999 (1999-06-10) claims 1-5,11 ---	1-3,7, 9-14,27, 28,30
X	EP 0 680 745 A (L'OREAL) 8 November 1995 (1995-11-08) claims 1,3,5-9,11,15 page 3, line 18-24 ---	1-4,7, 9-14,27, 30
X	WO 98 02137 A (INDUSTRIA E COMERCIO DE COSMETICOSNATURA) 22 January 1998 (1998-01-22) claims 1,2 --- -/--	1-3,7, 10,25-28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 May 2002	07/06/2002

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/US 01/30303

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 843 002 A (KAO CORPORATION) 20 May 1998 (1998-05-20) claim 1 page 4, line 42-45	1-14,27, 28,30
X	DATABASE WPI Week 198701 Derwent Publications Ltd., London, GB; AN 1987-003711 XP002199663 SHISEIDO: "Safe hair cosmetic for dandruff prevention-containing the extract of luffa cylindrica and e.g. zinc pyrithione, chlorhexidine gluconate etc." & JP 61 263910 A (SHISEIDO), 1987 abstract	1-5, 10-14,30
X	PATENT ABSTRACTS OF JAPAN vol. 0110, no. 85, 1987 & JP 61 238719 A (SHISEIDO), 1986 abstract	1-3, 10-14,30
X	DATABASE EPODOC 'Online! EUROPEAN PATENT OFFICE, THE HAGUE, NL; LEE YU-SOON: "Making-up soap composition" XP002199662 abstract	1-3,10, 30
X	& KR 9 604 499 A (LG CHEMICAL) 1996	1-3,10, 30

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int(onal Application No
PCT/US 01/30303

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9927792 A	10-06-1999	AU 741591 B2	06-12-2001
		AU 1476199 A	16-06-1999
		WO 9927792 A1	10-06-1999
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